EXHIBIT 13

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1: Insect Biochem Mol Biol. 2005 Apr;35(4):333-45. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Cloning and characterization of the G protein betagamma subunits from Trichoplusia ni (High Five cells).

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Baculoviral-mediated expression in insect cells has become a method of choice where high-level protein expression is desired and where expression in Escherichia coliform (E. coli.) is unsuitable. Genes of interest are inserted into the baculoviral genome of Autographa californica nuclear polyhedrosis virus (AcNPV) under the extremely strong, but very late polyhedron gene (PolH). The preferred host lines are derived from Spodoptera frugiperda (Sf9 or Sf21) or Tricoplusia ni (High Five, Invitrogen). Viral expression in insect cells is commonly used in the signal transduction field, due to the more than satisfactory capacity to express membrane proteins. However, co-association and/or co-purification of contaminating endogenous host G protein subunits, for example, may potentially threaten the functional and structural homogeneity of membrane preparations. The undefined G protein composition is complicated by the limited sequence data of either the S. frugiperda or Tricoplusia ni genomes. Here we report the isolation of cDNAs encoding two members of the heterotrimeric G protein family, Gbeta (Tn-Gbeta) and Ggamma (Tn-Ggamma), from Tricoplusia ni. Tn-Gbeta shares approximately 90% amino acid sequence identity with Gbeta from Drosophila melanogaster and 84% identity with mammalian Gbeta (human Gbeta1). Tn-Ggamma shares approximately 71% amino acid identity with D. melanogaster Ggamma1 and 42% identity with mammalian Ggamma (human Ggamma2). Tn-Gbetagamma is also functionally similar to mammalian Gbeta1gamma2 by virtue of their capacity to form a complex with mammalian Galpha subunits, support G-protein-dependent agonist binding to a mammalian G protein-coupled receptor (beta2-adrenergic receptor) and directly regulate effectors such as adenylyl cyclase.